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Bioefficacy of efficient entomopathogenic fungus against branch canker pathogen (*Macrophoma theicola*) in tea plantations of southern India

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ABSTRACT

Three branch canker pathogens, viz. NBCHE-6, UPA-61 and VPM were isolated from different tea growing districts of south India and four entomopathogenic fungus, viz. *Beauveria bassiana, Paecilomyces lilacinus, Lecannicillium lecannii* and *Paecilomyces fumosoroseus* were procured from the microbial type culture collection and gene bank (MTCC), Chandigarh. *In vitro* studies revealed that *Beuveria bassiana* showed highest antagonistic effect against NBCHE-6 (64.22) followed by *Paecilomyces fumosoroseus* against UPA-61(56.66). *Paecilomyces lilacinus* significantly controlled VPM (54.66), while *Lecannicillium lecannii* showed insignificantly control against VPM (47.33). While *Beuveria bassiana* and *Paecilomyces lilacinus* coiled around and shrink branch canker pathogen, *Lecannicillium lecannii* breaks into branch canker hyphae and *Paecilomyces fumosoroseus* produces more spore to kill branch canker. In culture filtrate studies, *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* showed maximum control of VPM (68.44) and UPA-61 (65.59). *Beauveria bassiana* also showed significant control of two isolates VPM and UPA-61 (54.44). *Lecanniicillium lecannii* showed least control of VPM (30.44). This study concludes that entomopathogens can significantly control branch canker pathogen (*Macrophoma theicola*).

Key words: Antagonist, Entomopathogens, Hyperparasitism, *Macrophoma* sp, Non-volatile culture filtrate, Tea

Tea, being a perennial crop, provides a stable environment for a number of pests and diseases. Tea plantations suffer heavily from the infestation. Pests, pathogens and weeds are important factors limiting the productivity and quality of processed tea. Stem diseases are important as they stagnate crop and sometimes kill the bush. Pruning operation in tea increases the risk of stem diseases since it exposes the wood tissue to parasitic cuts and saprophytic fungi. Branch canker in tea was first noticed in southern India in 1899, but in Srilanka the diseases was recorded in 1904 (Petch 1923).The pathogen *Macrophoma* sp. is a wound pathogen.The fungal pathogen can easily enter through prune cuts or tissues damaged by sun-scorch. The pruning cuts also provide ideal surface for germination of spores (Otieno 1997). Stem diseases like wood rot (*Hypoxylon serpens*), collar canker (*Phomopsis theae*), branch canker (*Macrophoma theicola*) and thorny stem blight (*Tunstallia aculeata*) are predominant in southern India.Recent researchers reported that, entomophathogen could be used as plant fungal disease. Entomopathogenic fungi such as *Lecanicillium* sp.and *Beauveria bassiana* have shown to engage in plant pathogenic fungi interaction (Vega 2008 and Vega *et al.* 2008).These entomopathogens have been reported to very effectively control plant disease (Goettel *et al*. 2008 and Ownley *et al*. 2008). The entomopathogenic fungi are considered integrated control for chewing and sucking insect and pest (Gallego and Gallego 1988).The entomopathogens are (*Paecilomyces fumosoroseus, Lecanicillium, Beauveria bassiana* and *Metarhizium anisopliae*) commercially developed as biopesticides (Goettel *et al*. 2005). Moreover, these entomopathogens have been confirmed against plant fungal pathogen (Kavkova and Curn 2005). In our attempt, performance of entomopathogens like *Beauveria bassiana, Paecilomyces fumosoroseus,Lecanicillium lecanii* and *Paecilomyces lilacinus* have been evaluated against *Macrophoma* sp. The studies were conducted under *in vitro* level.

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MATERIALS AND METHODS

The infected stem portions were collected from different tea growing district of south India, viz. Anamallais,

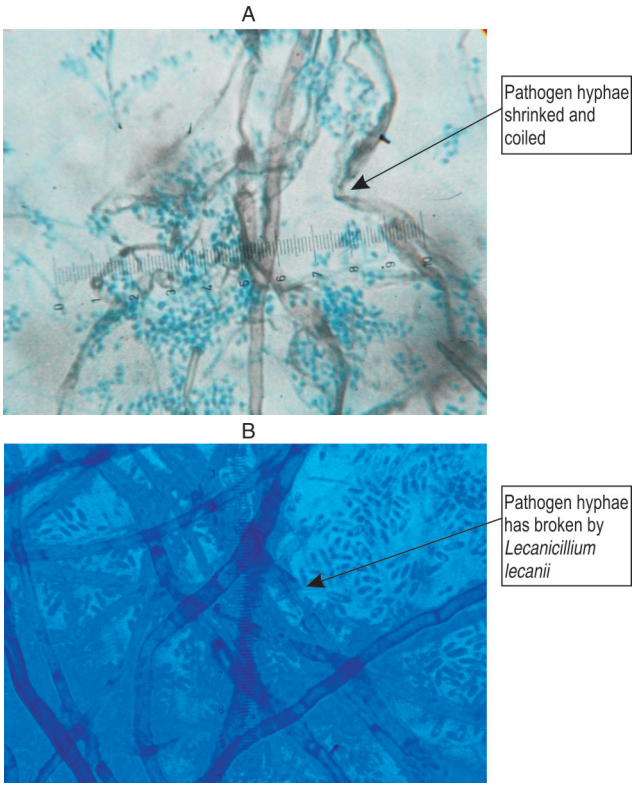

Fig 1 Hypal interaction between entomopathogen and pathogen (under 10X view)

Coonoor, and Vandiperiyar. The plant part were then examined under microscope.The fungus was morphologically and cultural characteristically identified following "The diseases of tea bush" (Petch 1923).Isolation of genomic DNA amplification (PCR) was performed to18srRNA gene of the fungus sequencing analyses were identical used by Crous *et al.*(1999).Total of three strains were isolated namely VPM, UPA-61and NBCHE-6.These fungal pathogens were confirmed as *Macrophoma* sp and *Macrophoma theicola* through molecular technique identifications and submitted to NCBI (NBCHE-6-Accession No. JQ234977, VPM-Accession No. KP004441 and UPA-61-Accession No. KP17922).

The potential of entomopathogens were screened against *Macrophoma* sp. branch canker pathogen by dual culture method as described by Rajendiran *et al*. (2010).The entomopathogens were procured from Microbial Type Culture Collection and Genebank (MTCC), Chandigarh, namely like *Beauveria bassiana, Paecilomyces fumosoroseus, Lecanicillium lecanii* and *Paecilomyces lilacinus*. The pathogen and antagonist were inoculated in PDA plates on diametrically opposite points. Since the entomopathogens were slow growing in nature, the antagonists were inoculated only before the pathogen colony grew considerably therefore, after 2 days. Linear growth of the biocontrol agents colonizing either over or meet each other the pathogens growth was measured after 9 days of incubation. For the testing of antagonistic entomopathogens *Beauveria bassiana, Paecilomyces fumosoroseus, Lecanicillium lecanii* and *Paecilomyces lilacinus* 6 mm discs of antagonist and *Macrophoma* sp cut from the edge of 7 days old culture were placed 3 cm apart on potato dextrose agar (PDA) plate. The Petri plates were incubated at 27 ± 1ºC and periodical observations on the growth of the antagonist to colonize the pathogen were recorded. The experimental design used was completely randomized with four dishes for each isolate and control plate (without entomopathogen), a sterilized agar disc plate. Antagonistic activity was measured using Bell'scale method (Bell 1982).The percentage of inhibitions was calculated by the formula, PI=C-T/C×100.PI-percntage of inhibition,C-radial growth of the pathogen in control, T- radial growth of the pathogen in dual culture.

The entomopathogen and test pathogen at the opposite edges and were incubated for 7-9 days and interaction between the opposing cultures including hypal contact or coiling and lysis, which was observed under the microscope. Hyphal interaction gently from the zone of interaction in dual culture plates with the help of a needle and placed in a drop of lactophenol cotton blue on a microscopic slide (Elad *et al*. 1983).

The effect of culture filtrate of entomopathogen was studied following the method of Dennis and Webster (1971). The entomopathogens were inoculated in 100 ml sterilized Potato dextrose broth in 250 ml conical flasks and incubated at 27 ± 1ºC .The liquid culture medium was filtered through Whatman No.1. The filtrate was centrifuged at 10000 rpm for 15 min. The supernatant was filtered using millipore membrane filter paper (0.22µm).The entomopathogen filtrate was added to molten PDA to obtain final concentration of 2% (v/v). The medium was poured into petri plates (20 ml/plate) and plates were inoculated with 6 mm disc of test pathogens. PDA plates inoculated with *Macrophoma* sp.but amended with sterile distilled water served as control. The plates were incubated at 27 ± 1ºC for 6 days.The percentage of inhibitions was calculated by the above formula.

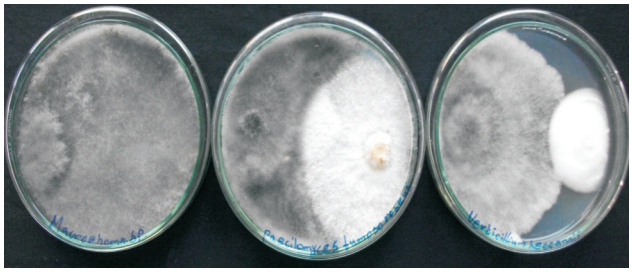

Fig 2 Growth inhibition of *Macrophoma* sp. by entomopathogen (*Paecilomyces fumosoroseus* and *Lecanicillium lecanii*)

Table 1 Isolation of branch canker pathogen from tea growing districts of southern India

Strain code No	Location	Identified as	NCBI Gen Bank Accession Number
NBCHE-6	Coonoor	*Macrophoma* sp	JQ234977
VPM	Vandiperiyar	*Macrophoma*	KP004441
UPA-61	Anamallais	*Macrophoma theicola*	KP179221

Table 2 *In vitro* screening of entomopathogen against branch canker pathogen (Dual plate technique)

Entomopathogen culture (MTCC)	Growth inhibition (%)		
	Macrophoma sp (NBCHE-6)	*Macrophoma* sp (VPM)	*Macrophoma theicola* (UPA-61)
Beauveria bassiana	64.22	61.55	62.66
Lecanicillium lecanii	45.33	47.33	44.22
Paecilomyces lilacinus	47.77	54.66	51.55
Paecilomyces fumosoroseus	54.44	56.66	56.66
CD (P=0.05)	3.1	2.8	2.8

Values are means ± SE of four replication of three repeated experiments.

Table 3 *In vitro* screening of entomopathogen against branch canker pathogen (Non-volatile culture filtrate)

Entomopathogen culture filtrates at 2% concentrations (MTCC)	Growth inhibition (%)		
	Macrophoma sp (NBCHE-6)	*Macrophoma* sp (VPM)	*Macrophoma theicola* (UPA-61)
Beauveria bassiana	53.33	54.44	54.44
Lecanicillium lecanii	18.55	30.44	26.55
Paecilomyces lilacinus	57.77	63.1	65.59
Paecilomyces fumosoroseus	60.04	68.44	66.77
CD (P=0.05)	7.1	4.9	4.4

RESULTS AND DISCUSSION

In this experiment results revealed that, the different tea growing area infected branch canker specimens were isolated and identified at molecular level. The details of branch canker isolate NBCHE-6,VPM and UPA-61 with source of location is given (Table 1). The entomopathogens have been tested for inhibition of branch canker pathogen.The inhibitory effects observed in this study were mainly for antagonistic and competition.

The dual plate method showed maximum growth inhibition of *Beauveria bassiana* against branch canker isolates NBCHE-6 (64.22), UPA-61 (62.66) and VPM (61.55). The effect of *Paecilomyces fumosoroseus* against two isolates of VPM and UPA-61 (56.66) and *Paecilomyces lilacinus* against VPM (54.66) was significantly antagonistic potential followed by *Lecannicillium lecannii* observation against VPM (47.33),NBCHE-6 (45.33) and UPA-61 (44.22) (Table 2 and Fig 2).

Mycoparasitism of both hypal interaction vital role in mechanism of antagonistic potential capability. The hyphae of *Beauveria bassiana* and *Paecilomyces lilacinus* coiled around hyphae of *Macrophoma* sp. and shrunk it (Fig 1A). However, same action *Beauveria bassiana* with pathogen of tomato root-rot *Pythium myrotylum*was reported by Klingeman *et al*. (2008). Kiss (2003) reported that, this *Beauveria bassiana* may control plant pathogens and can act through antibiosis and mycoparasitism. Pathogen hyphae was broken by *Lecannicillium lecannii* spores interaction (Fig 1B). The result conform to the reports of Askary *et al*. (1997) who reported that, *Lecannicillium lecannii* acts as mycoparasite attaching to powdery mildew mycelia and conidia, producing enzymes such as chitinase, which penetrates to the mildew spore and kills the pathogen. *Paecilomyces fumosoroseus* produced more hyphae and spores interact with pathogen hyphae. Several scientists reported the mycoparasitism interaction as main principle mechanism of biological control (Elad *et al*. 1983). This study results are in accordance with the reports of Kang *et al*. (1996), Verharr *et al*. (1997), Dik *et al*. (1998), Miller *et al*. 2004 and Kavkova and Curn (2005). This may also be the reason for its antagonistic effect on *Macrophoma* sp.

Culture free extract of entomopathogen namely, *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* have showed maximum inhibition in growth of the pathogen at 2% concentrations. The maximum inhibition given by *Paecilomyces fumosoroseus* against VPM (68.44) followed by *Paecilomyces lilacinus* against UPA-61 (65.59). Significant inhibition observed by action of *Beauveria bassiana* against both isolates of VPM and UPA-61(54.44). *Lecannicillium lecannii* was seen to inhibit VPM sparsely (30.44) as compared to other antagonistic treatments (Table 3).

The present studies reveal that, *Beauveria bassiana* and *Paecilomyce fumosoroseus* show higher control growth of *Macrophoma* sp. pathogen. Youssef and Hatem (2012) also reported the control of red palm weevil and *Rhizoctonia* –root-rot of date-palm with *Beauveria bassiana*. *Isaria fumosoroseus* (formely *Paecilomyces fumosoroseus*) and *Lecannicillium* sp (formely *Verticillium lecannii*) are known entomopathogens and have good biopesticidal properties (Goettel *et al*.2005). Wherever, these kind of entomopathogens against fungal plant pathogens (Benhamou and Brodeur 2000, 2001). In our findings, we conclude that *Paecilomyces lilacinus* showed good antagonistic result. Similar findings were recorded by Perveen *et al*. (1998) with *Paecilomyces lilacinus* and *Pseudomonas aeruginosa* when used against root rot (*Meloidogyne javanica*) and root knot disease (*Macrophomina phaseolina*) in some vegetable crops. The applications of *Paecilomyces lilacinus* fungus protect plant roots from pathogens, increase plant growth and leaf yield (Manjula and Podile 2001. Wraight *et al*. 2003 and Muthulakshmi *et al*. 2010).

Results of our experiment showed low inhibitory effect of *Lecannilium lecanni*, though, these entomopathagenic fungus have activity against phytopathogenic fungi including powdery mildew (Verhaar *et al*. 1997, 1998), Spencer and Atkey 1981, Askary *et al*. 1998). In the present investigation, all the entomopathogens studied, showed antagonistic potential and inhibitory effect against *Macrophoma* sp. pathogen. The evidence for role of competition and parasitism has been convinced and evidence established the importance of antibiosis.

From this study, it is evident that the entomopathogens reduced the growth of all isolates of branch canker pathogen causal organism by *Macrophoma theicola* significantly. Mostly entomopathogen can be used as pest and insect infection disease control. Hence, it may use in integrated approaches for managing plant disease and pest control.

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